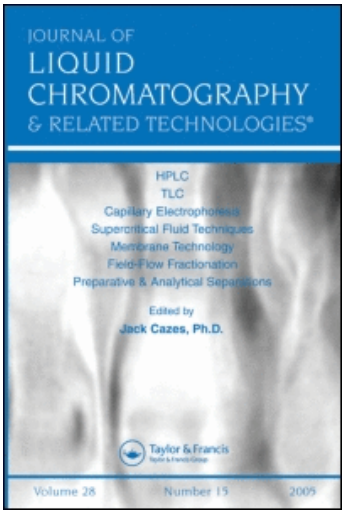

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

A Rapid Method for the Prefractionation of Essential Oils. Application to the Essential oil of Black Spruce [Picea Mariana (Mill.) BSP.]

Selma Hajji[a]; Jacques Beliveau[a]; David Z. Simon[a]; Romano Salvador[a]; Claude Aube[b]; Alberto Conti[b]
[a] Faculty of Pharmacy, University of Montreal, Montreal [b] Station de Recherches, Agriculture Canada, Que.

To cite this Article Hajji, Selma , Beliveau, Jacques , Simon, David Z. , Salvador, Romano , Aube, Claude and Conti, Alberto(1984) 'A Rapid Method for the Prefractionation of Essential Oils. Application to the Essential oil of Black Spruce [Picea Mariana (Mill.) BSP.]', Journal of Liquid Chromatography & Related Technologies, 7: 13, 2671 — 2677
To link to this Article: DOI: 10.1080/01483918408067034
URL: http://dx.doi.org/10.1080/01483918408067034

JOURNAL OF LIQUID CHROMATOGRAPHY, 7(13), 2671-2677 (1984)

A RAPID METHOD FOR THE PREFRACTIONATION OF ESSENTIAL OILS. APPLICATION TO THE ESSENTIAL OIL OF BLACK SPRUCE [PICEA MARIANA (MILL.)BSP.]

SELMA HAJJI,JACQUES BELIVEAU,DAVID Z.SIMON,ROMANO SALVADOR
Faculty of Pharmacy,University of Montreal,Montreal.

CLAUDE AUBE,ALBERTO CONTI
Station de Recherches,Agriculture Canada,St.Jean,Que.

ABSTRACT

A rapid method for the prefractionation of complex essential oils, prior to the identification of their components,is described.The use of "hybrid" flash chromatography,developed in our laboratories,in a specially constructed,jacketed column,which permits the use of low boiling solvents,makes it possible to effect satisfactory separations through a single column rather than through multiple columns as in classical methods,and in a fraction of the time required.The procedure enables a facile separation of hydrocarbons from oxygenated compounds and yields,in some instances,fractions of pure compounds.Enriched fractions of trace constituents are also obtainable for identification by gas chromatography.The method is rapid and inexpensive.

INTRODUCTION

Essential oils are complex mixtures of terpenoids and phenylpropane derivatives.The number of components in any single oil may vary from few to over one hundred.The presence of that many individual components makes the analysis of the oil,by gas chromatography,very difficult because of overlap of peaks and similarity of retention times.Thus,prefractionation of the oil,prior to analysis is of utmost importance.

Various methods of prefractionation have been described, most using multiple columns.To our knowledge,Scheffer et al.

 0148-3919/84/0713-2671$3.50/0

(1,2)have developed one of the best fractionation techniques, involving liquid-solid chromatography,but their method requires two columns for the separation of hydrocarbons from oxygenated components,the gel must be specially treated and a mixture of gels of various sizes is required for the separation of each fraction.The procedure is time consuming varying from days to weeks in certain cases.The procedure we will describe permits a separation similar to that of Scheffer in only 45 to 60 minutes.

The basis of the process was described by Still et al.(3). In this method,called "flash chromatography" a column,the diameter of which varies with the quantity to be separated,is filled to 15 cm with silica gel(40 - 63 μm).The column is fitted, at its top,with a relief valve through which a flow of nitrogen is introduced.The pressure is regulated by means of the relief valve so that the elutant is forced through the column at the rate of 2.5 cm per minute.The composition of the elutant is that which gives a separation on a silica plate in TLC.This type of "three dimensional" TLC separates the mixture in 45 to 60 minutes,the separation following closely the Rf values on TLC.

We have combined the essentials of "flash chromatography" with the procedure of Scheffer to make possible the superior separation obtained by Scheffer in only 45 to 60 minutes on a single column.This technique,which we term "hybrid" flash chromatography,has made it possible to separate complex mixtures, to isolate some of the constituents in the pure state and to obtain enriched fractions of trace constituents in a very short time.In essence we have combined a gradient technique,similar to that of Scheffer,with the "flash chromatography"of Still.The order of elution,for the essential oils,is identical to that described by Scheffer.The hydrocarbons are first eluted with hexane and the remaining components are eluted with an increasing gradient of ether in hexane.The oxygenated compounds are eluted in the following order according to the functional group:oxides,

esters,ketones and alcohols.Using different solvent mixtures we have also applied this technique to the separation of alkaloids (4) and saponins(5).

EXPERIMENTAL

Instruments and Operating Conditions

The column for "hybrid" flash chromatography is a standard glass column,equipped with a water-cooled jacket,for its entire length,to allow the use of low boiling solvents.The nitrogen flow was regulated by the relief valve to force a drop of 2.5 cm per minute of the surface of the elutant.The construction of the relief valve was as described by Still.

Gas chromatography was done on a Perkin-Elmer Model 990, modified to accept a 60 m capillary column(Durawax fused silica; J&W DX4-60N) and FID.Nitrogen gas flow was 16.6 cm/sec.,with a 50:1 split ratio.The program was 90^{o} to final temperature 180^{o} at 3^{o}/min. for the whole oil and for the hydrocarbons.For oxygenated compounds the program was 130^{o} to final temperature 180^{o} at 4^{o}/min.Injector and manifold temperature as well as the FID was held at 250^{o}.

The spruce oil was extracted by "hydrodiffusion" using a Shmid Hydrodiffuser(Switzerland) Model LS-500,kindly provided by PAL Hydrodiffusion(Canada) Inc., Ste.Adele, Quebec,Canada.

Materials and Standards

Hexane(Fisher HPLC grade) and ether for anesthesia(Biopharm) were used as eluting solvents.Silica Gel 60(E.Merck No.9385 200-400 mesh) was heated at 105^{o} for 60 minutes and deactivated by addition of 5% water.

Ten standards were used:-bornyl acetate,limonene,myrcene, p-cymene,cineole,αterpineole,carvone,linalool,menthol(Aldrich Chemical) and fenchone(Fluka).

The black spruce oil was extracted in our laboratories,by hydrodiffusion,from identified species collected in March 1983 in the region north of Montreal(the Laurentides).

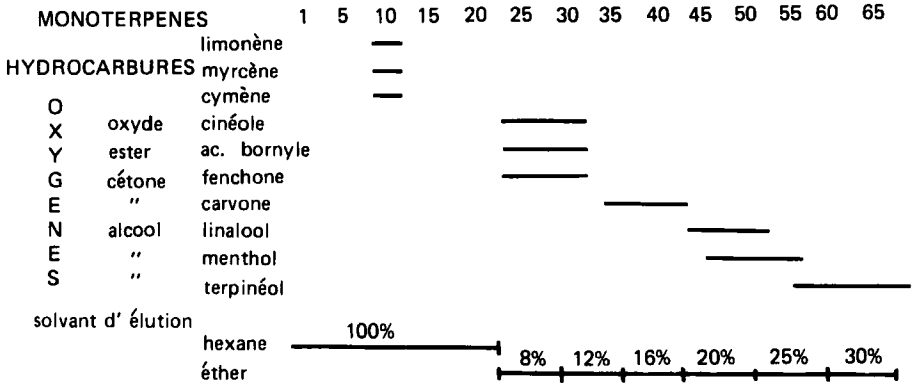

FIGURE 1 : Elution of test mixture showing separation of hydrocarbons from oxygenated compounds. Total time:- 55 minutes.

TABLE 1

Main Constituents of Black Spruce Oil Separated by "Hybrid" Flash Chromatography

Constituent	RT Capillary Column	Peak No.	Percentage %
α-pinene	9' 40	3	13.8
Camphene	11' 20	4	24.9
β-pinene	12'	5	4.1
myrcene	13' 40	6	0.1
limonene	15' 40	8	0.5
1,8-cineole	16' 10	9	4.9
bornyl acetate	36' 40	19	41.2
borneol	43'	29	trace
piperitone	44' 40	32	trace

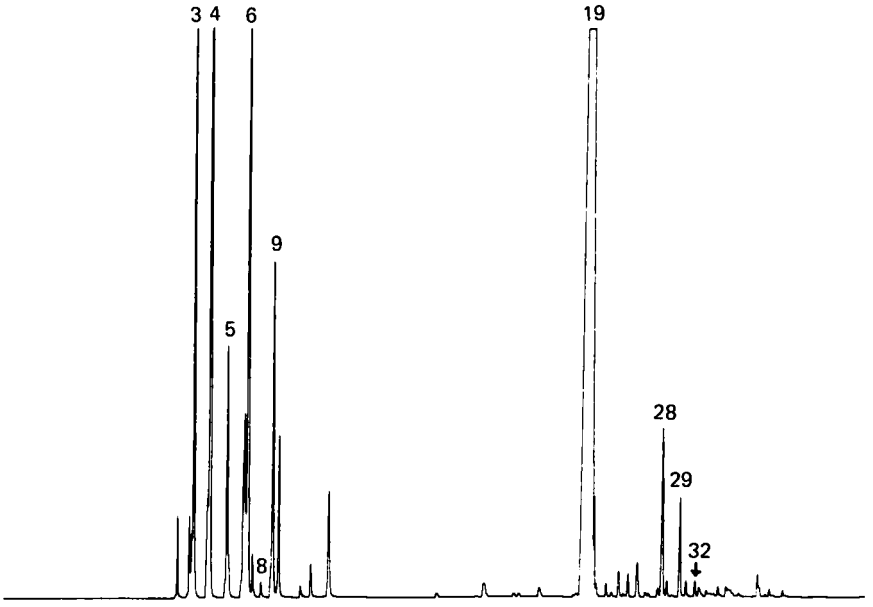

FIGURE 2 : GLC spectrum of Oil of Black Spruce.Numbered peaks are identified in Table 1.

PROCEDURE

To verify the efficacy of the "hybrid" flash chromatography procedure,a test mixture was separated prior to its application to spruce oil.

Preparation of Test Mixture

Each of the standards was dissolved in hexane as follows: carvone 7%;limonene,myrcene,cymene,cineole and terpineol 9%; bornyl acetate and fenchone 10%;linalool and menthol 12%.

A mixture of 0.2 ml of each(total 2 ml) was applied to the column containing 50 g(15 cm)of gel.

Separation of Test Mixture

The column,containing 2 ml of the test mixture was eluted first with hexane under nitrogen pressure sufficient to force a drop in solvent level of 2.5 cm/min. Twenty-two fractions of 10 ml each were taken.Nitrogen pressure was removed and the hexane was replaced with a mixture of 8% ether in hexane.The pressure was restored and seven fractions of 15 ml were taken.

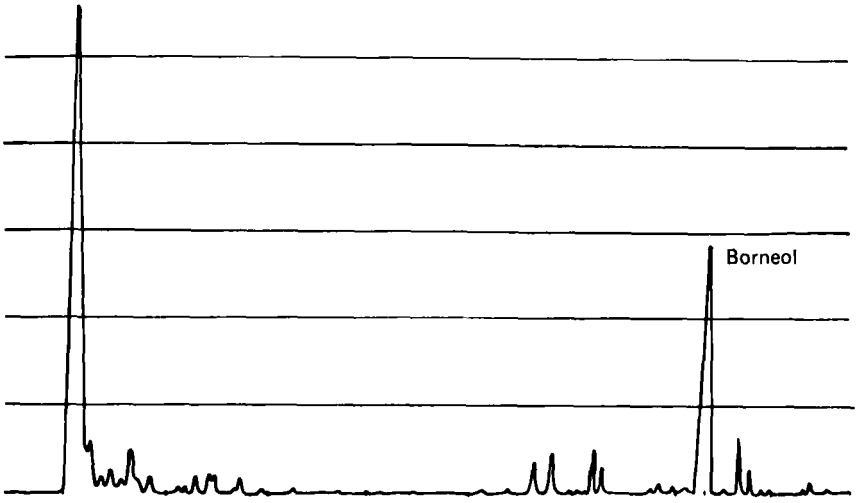

FIGURE 3 : Peak 29--Borneol.An enriched fraction.Compare with this same peak in Figure 2. (GLC)

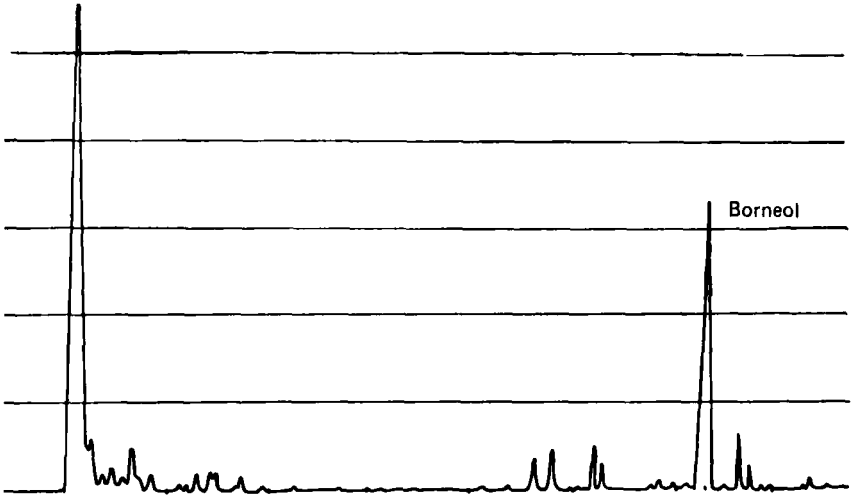

FIGURE 4 : Peak 29--Borneol.Identification by peak enrichment in GLC.

Similarly seven fractions of 15 ml were taken at each increased concentration of ether in hexane i.e. 12%,16%,20%,25%,30% and 50%.The fractions were evaporated to 1 ml for analysis by gas chromatography.The order of elution is the same as that reported by Scheffer(2).(Figure 1)

Chromatography of Black Spruce Oil

The separation of 2 ml of black spruce oil was done on the same column under identical nitrogen pressure and identical gradient,the same number of fractions of the same volume being taken. The entire separation was completed in 55 minutes.The fractions isolated were identified by gas chromatography by means of retention times and peak enrichment.The main constituents are reported in table 1 while the entire spectrum is shown in Figure 2. The identification of peaks 29 and 32 of Figure 2 borneol and piperitone respectively,both present in trace amounts,was made simple by combining fractions of several runs and thus obtaining enriched fractions which were used for the identification.The speed of the prefractionation makes multiple runs not at all tedious. (Figures 3 and 4)

CONCLUSION

The application of the gradient technique to "flash chromatography provides a very rapid means for the separation of complex mixtures,with resolution as great or greater than the time consuming classical methods.Repeated runs for fraction enrichment are no longer to be dreaded.Equipment is not costly and unless low boiling solvents are to be used,an ordinary column fitted with a relief valve,as described by Still,and silica 200 - 400 mesh is all that is required.

REFERENCES

1 Scheffer,J.J.C.,Koedam,A. and Svendsen,A.B.,Chromatographia, 9, 425 (1976)

2 Scheffer,J.J.C., Koedam,A.,Shusler,M.Th.I.W. and Svendsen, A.B., Chromatographia, 10, 669 (1977)

3 Still,W.Clark, Kahn,M. and Mitra,A., J.Org.Chem., 43, 2923 (1978)

4 Eloumi-Ropivia,J.,Beliveau,J. and Simon,D.Z., Master thesis of J.Eloumi-Ropivia.

5 Tedeneke,W.,Beliveau,J. and Simon,D.Z. Masters project of W.Tedeneke